Quantitative Analysis of Dose-Response Data Obtained With Three Carcinogenic Hydrocarbons in Strain C3H Male Mice

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In a previous communication (*1*) analyses were made of published data dealing with the responses of mice to measured quantities of several carcinogenic hydrocarbons. Certain conclusions were reached regarding the nature of the latent-period and percentage response curves obtained with such agents. This paper deals with investigations carried out to test further these conclusions and to compare the results obtained in simultaneous studies of 20-methylcholanthrene, 1, 2, 5, 6-dibenzanthracene, and 3, 4-benzpyrene.[1] The former conclusions are substantiated, and estimates are made of the relative carcinogenic potencies of the above-named materials.

EXPERIMENTAL PROCEDURE

The corrected melting points of the three hydrocarbons used were: methylcholanthrene, 179.7°–180.2° C.; dibenzanthracene, 267.3°–267.7°; benzpyrene, 178.2°–178.9°.[2] The same batches of the chemicals were used throughout the investigation.

The hydrocarbons were dissolved in tricaprylin with a melting point of 8.3°–9.0°. The various solutions used for injection were prepared by making appropriate dilutions of standard stock solutions of the hydrocarbons so that the unit volume of solvent containing the quantity of hydrocarbon injected was 0.25 cc., except in two dose groups, noted in the tables, where 0.5 cc. of solvent was used. The suspensions containing the higher concentrations were prepared by weighing separate batches of the hydrocarbons for each dose and adding the desired amount of solvent.

The doses were spaced logarithmically over a wide range (0.00024 to 8.0 mg.) in order to include extreme responses and thus obtain information regarding the range of doses within which both the incidence and the latent period of tumor response are correlated with dose.

The test animals were 1,004 male mice of strain C3H, which were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and which were received in varying lots during the first 3 months of 1941. Each lot was divided at random into 3 groups for injection with the respective hydrocarbons. Subdivision of the groups according to the various doses of the 3 chemicals was also carried out. The mice were 1.5 to 3 months old, and most of them weighed 20 to 24 gm. at the time of injection. They were maintained on unlimited supplies of water and Purina dog chow, and were individually identified.

All injections were made into the sub-

[1] *Hereinafter referred to as methylcholanthrene, dibenzanthracene, and benzpyrene.*

[2] *The hydrocarbons and the solvent were supplied by Dr. J. L. Hartwell, of the National Cancer Institute.*

cutaneous tissues of the right axilla. In order to prevent leakage, a long needle was inserted just above the tail in the dorsal region and passed diagonally through the subcutaneous tissues into the right axillary space. The volumes injected were measured with tuberculin syringes of 0.5-cc. capacity.

Examination, by palpation, of each individual mouse for subcutaneous tumor was carried out every fourth or fifth day beginning 4 weeks after injection. Mice with subcutaneous nodules thought to be early tumors were isolated at each examination, and the growth of the nodules was followed every second day by measurement until the growth had reached a mean diameter of about 20 mm. unless death intervened. Sections through several planes were taken of the tumor tissue at necropsy for microscopic examination.

A progressively growing tumor when proved by subsequent growth and histologic appearance to be a malignant neoplasm, was listed as having appeared on the date on which the nodule was first recorded. The interval between injection and appearance of the tumor was considered the latent period.

Two factors may complicate the first detection of a tumor nodule. The first is the formation of cysts containing the injected solvent. These cysts are ruptured by slight pressure and can be ruled out easily (*2*). The second factor is the thickening of the subcutaneous tissue which occurs with higher concentrations of the hydrocarbons (*3*). Occasionally the thickening may be nodular because of small encapsulated areas of necrosis or oil. This reaction appears fairly soon after injection and usually subsides before the appearance of a progressively growing malignant process.

Of the 1,004 strain C3H male mice originally employed, 957 contributed to the final analysis of results. Forty-seven animals were listed as missing or dead and autolyzed before small nodules were conclusively diagnosed as tumors and were discarded from the results.

A total of 433 tumors [3] at the site of injection was observed in the 957 mice. The morphology of 415 tumors that were examined histologically is described and analyzed in another publication (*4*). Of the 415 tumors, 411 (99 percent) were spindle-cell sarcomas, 2 were carcinomas, and 2 consisted of mixed sarcoma and carcinoma elements. The 18 tumors that have been included, in the absence of histologic examination, grew rapidly to a fairly large size (10 to 20 mm. in diameter) but were lost for microscopic studies because of death and autolysis or because they were devoured by other mice.

RESULTS

The results for the different hydrocarbons are summarized in tables 1, 4, and 7, respectively, which give as much information as is practicable concerning the actual data. Detailed information in regard to individual latent periods for the respective hydrocarbons is given in the scatter diagrams of figures 1, 9, and 18.

Analyses were made to determine the variability of the biologic responses and to determine the trends of the various group responses with dose of hydrocarbon. In addition, certain statistics were calculated to facilitate comparison between the results obtained with the various carcinogenic agents as well as with data from the literature. For simplicity, graphic presentation of the analyses is given where practicable. Most of the biomathematical procedures involved in these analyses are discussed in detail by Bliss (*5*, *6*, *7*) and

[3] *A few mice developed multiple tumors, but only the first tumor occurring in a given animal is considered in this report.*

Irwin (*8*). Where tests for statistical significance were carried out, the results are indicated in the text as follows: statistically significant, when P is less than 0.01; not statistically significant, when P is greater than 0.05; probably significant when P is between 0.01 and 0.05. Values preceded by a $\pm$ sign represent standard errors. Limits of error corresponding to a probability of 0.05 are designated where included.

Methylcholanthrene

The results with methylcholanthrene are summarized in table 1. The upper limit, 1.0 mg., of the dose range (0.00024 to 1.0 mg.) was selected because previous studies (*9*), in which methylcholanthrene had been administered to similar test animals under similar conditions, had showed that the incidence of tumors was 100 percent at all doses above 0.25 mg. Furthermore, above this dose level the latent period was constant and unrelated to dose.

TABLE 1.—*Summarized results on latent period of tumors and tumor incidence of mice injected with methylcholanthrene*

Dose [1]		Mice injected	Mice with tumors	Latent period			Tumor incidence			
				Mean latent period [2]	Standard deviation [2]	Weight of observation [3]	"Corrected total"	Mice with tumors	Probits [4]	Weight of observations
Mg.	*Log mg.*	*Number*	*Number*	*Months*	*Months*		*Number*	*Percent*		
1.0	0.000	20	20	2.40	0.40	20	20	100		
0.5	−.301	21	21	2.57	.40	21	21	100	[5] 8.484	0.063
0.25	−.602	21	21	2.77	.61	7.64	21	100	[5] 7.860	.441
0.125	−.903	21	21	3.32	.92	3.72	21	100	[5] 7.268	1.911
0.062	−1.204	21	17	3.92	1.01	1.78	19.7	86.3	6.094	7.98
0.031	−1.505	20	13	5.24	1.87	.90	19.9	65.3	5.393	11.96
0.0156	−1.806	18	6	4.59	.96	.29	16.5	36.4	4.652	9.97
0.0078	−2.107	17	3	6.97	4.64	.11	15.8	19.0	4.122	7.55
0.0039	−2.408	19	0				[6] 14.6	0	[5] 2.893	1.372
0.00195	−2.709	19	0				[6] 16.6	0	[5] 2.319	.578
0.00098	−3.010	41	0				[6] 29.2	0	[5] 1.695	.175
0.00024	−3.612	79	0				[6] 33.6	0		

[1] The volume of solvent used for each injection was 0.5 cc. at dose 1.0 mg.; 0.25 cc. at all other doses.
[2] Months=Days/30.
[3] See text.
[4] See Bliss (*5, 7*).
[5] Estimated probits for 0- and 100-percent responses.
[6] Provisional values determined by extrapolation.

Latent Period

Standard deviation.—The latent periods are shown graphically in figure 1, in which they have been plotted against the logarithm of dose. The graph shows the variation of the latent periods at successive dose levels, i.e., the vertical spread of the plotted points. The horizontal and oblique lines represent calculated values and are for the purpose of showing the estimated vertical distribution at continuous dose levels. They are in terms of accumulative percentages of mice (within the tumor population only) which would be expected to develop tumors at or prior to the times indicated along the ordinate (fig. 1). For example, the top line of the figure shows for any given dose the time at which 99 percent of the mice that are going to develop tumors would be expected to have developed them. The derivation of the calculated lines is discussed later (p. 509).

In figure 1 it will be noted that the latent periods are most stable at the two highest doses (0.5 and 1.0 mg.), but that their variability increases as the dose becomes smaller.

The standard deviation of individual latent periods about their respective group

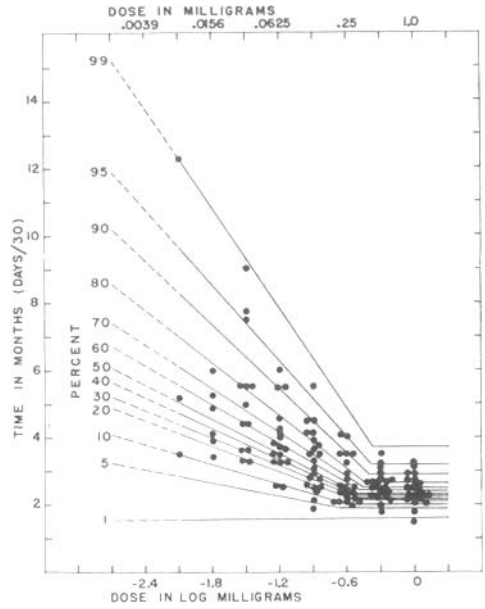

FIGURE 1.—Latent periods of tumors induced with methylcholanthrene. The solid circles represent individual observations plotted to avoid superposition of points at given doses. The oblique and horizontal lines show the calculated variation at continuous dose levels.

means are given in table 1 and shown graphically in figure 2. Their regression on log dose is statistically significant and

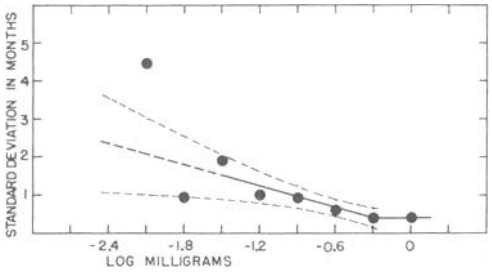

FIGURE 2.—Standard deviation of latent periods with successive doses of methylcholanthrene.
Circles: Observed standard deviations;
Solid line: Calculated regression line;
Curved dash lines: Limits of error of calculated regression line for $P=0.05$.

may be adequately represented as linear within the limits indicated by the solid oblique line. The equation to the regression line is:

$$\sigma_c = 0.584 - 0.950\ (X + 0.519) \qquad (1)$$

where σ_c is the calculated standard deviation in months (days/30) and X the dosage in log milligrams. The curved lines[4] show the limits of error of the calculated regression line corresponding to $P=0.05$. The wide range of variation coupled with a low incidence of tumors at the low dose levels make estimates of the standard deviation in this region of little practical importance unless very large numbers of animals are used. The standard errors of the estimates for the two lowest doses (0.015 and 0.0078 mg.) are so large that the information which they contribute with respect to the true nature of the trend is of no consequence. The regression in this region is therefore indicated as a broken line which should be considered only as a provisional estimate and not as definite information regarding the exact nature of the trend.

On a basis of previous studies (*9*) in which the value of the minimal standard deviation was identical with that reported here, 0.4 months (days/30), the standard deviation is considered to have reached its minimal level at about dose 0.5 mg. (−0.301 log mg.). That the standard deviation of individual latent periods would not be decreased below this level by larger doses of methylcholanthrene is further indicated by the breaks in the trends of results shown in figures 1 and 2.

Relative weights of latent-period observations at different dose levels.—The reliability of a latent-period response, such as a group mean, is dependent upon the variability as well as upon the total number of individual observations employed in its derivation. In the following analyses, the

[4] *The curved lines of this and subsequent figures represent the limits within which the relation between dose and response has been established. They apply to the calculated regression line and not to the distribution of points about the calculated line.*

reciprocal of variance ($1/\sigma^2$) was used as a weighting factor for adjusting the weights of responses at different dose levels with respect to the variability factor.[5]

Table 2, column 5, shows the relative weights when the number of observations (mice with tumors) is constant and when the maximum weight for any one dose group is considered as unity. The maximum weight is reached with those doses that yield the minimal standard deviation. The relative weights may be employed as weighting coefficients, and they may also be used as a basis for estimating the numbers of unit observations required to give results possessing the same degree of reliability at different levels of dosage. Column 6 shows the relative numbers when 10 are considered at the dose level where the weight is at its maximum. It should be emphasized that the relative numbers in this column refer to individual latent-period determinations and that they represent, therefore, only mice that actually develop tumors. The incidence of tumors decreases with the dose of methylcholanthrene, and it is necessary to increase accordingly the total number of injected mice to produce the desired numbers with tumors indicated by column 6. (See section entitled "Joint Use of Latent Period and Tumor Incidence Data," also columns 7 and 8 of table 2.)

[5] *This procedure was suggested by Dr. Harold F. Dorn, Division of Public Health Methods, National Institute of Health, to compensate for the correlation between standard deviation and dose. In the present analyses, the calculated standard deviations, derived from equation (1), were used rather than the observed standard deviations. The maximum value of $1/\sigma_c^2$ was considered as unity for determining the relative weights, or weighting coefficients, used in the analyses.*

TABLE 2.—*Relative weights of latent-period observations and numbers of mice required for observations of equal weight at various doses of methylcholanthrene*

Dose (in milligrams)	Expected mean latent period [1]	Expected standard deviation [1] (σ_c)	Reciprocal of variance ($1/\sigma_c^2$)	Relative weight (weighting coefficient) [2]	Mice with tumors required for responses of equal weight	Expected incidence of tumors	Total injected mice required for latent period responses of equal weight
	Months	*Months*			*Number*	*Percent*	*Number*
1.0	2.48	0.40	6.25	1.0	10	100.0	10
0.5	2.48	.40	6.25	1.0	10	100.0	10
0.25	2.73	.66	2.27	.364	27	99.6	27
0.125	3.40	.95	1.11	.178	56	97.3	58
0.0625	4.07	1.23	.656	.105	95	88.1	108
0.0312	4.74	1.52	.432	.069	145	66.8	217
0.0156	5.42	[3] 1.81	[3] .306	[3] .049	[3] 204	37.9	[3] 538
0.0078	6.09	[3] 2.09	[3] .228	[3] .036	[3] 278	14.6	[3] 1,904
0.0039	6.76	[3] 2.38	[3] .177	[3] .028	[3] 357	3.6	

[1] Months = Days/30.
[2] See text.
[3] Provisional values determined by extrapolation.

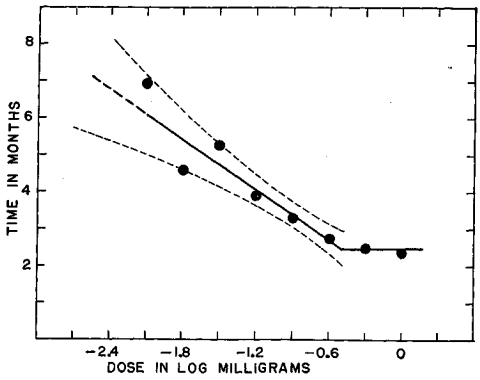

FIGURE 3.—Dose response curve for methylcholanthrene showing regression of mean latent period on log dose.
Circles: Observed mean latent periods;
Solid line: Calculated regression line;
Curved dash lines: Limits of error of calculated regression line for $P=0.05$.

Mean latent period.—Statistical analyses [6] indicate that the regression of mean latent period on log dose is significant when dosages of methylcholanthrene smaller than 0.5 mg. (−0.301 log mg.) are used.

The regression (below dose −0.3 log mg.) is adequately represented by the oblique line of figure 3, the equation to which is:

$$Y=3.274-2.233\ (X+0.846) \qquad (2)$$

where Y is the estimated value of the mean latent period in days/30 and X the dosage in log milligrams.

The latent period is considered to have reached a minimal level with the two highest doses, 0.5 and 1.0 mg. (−0.3 and 0 log mg.). This is indicated by the apparent break in the trend of responses at this dose level (figs. 1 and 3) and by the results of a previous study (*9*) in which the established minimal latent period, 2.27 days/30, was very nearly the same as that found here, 2.48 days/30.

Frequency distribution of individual latent-period observations.—Exact information regarding the distribution of individual latent periods about the true response curve for methylcholanthrene cannot be derived from data available at the present time. However, an approximation made from the distribution of observed latent periods about their respective group means may be of value for certain practical purposes such as the weighting of animals that die without tumors, and estimation of the time-tumor frequency relationship at various dose levels.

In a previous communication (*1*) the frequency relationships of the normal curve were used for weighting animals that died without tumors.[7] It would be more desirable, however, to use the actual frequency distribution if this were known. Figure 4 shows the distribution of latent periods obtained by compiling the data of the present experiments and those of Shimkin (*11*) to give a total of 198 unit observations suitable for the analysis. Combination of the data for various doses and the different experiments was made possible by expressing the individual latent periods as deviations from their group means in standard deviation units.

It is apparent from figure 4 that the frequency distribution is fundamentally skewed, indicating an excess of values in

[6] *The finding in a previous section of a significant correlation between the standard deviation of individual latent periods and the logarithm of dose necessitates a modification of the statistical procedures described by Irwin* (8) *for analyses involving the continuous type of variate. Irwin treated only the condition in which the standard deviation is the same at all dose levels but pointed out that, if this were not the case, special procedures would have to be introduced. It is beyond the scope of the present investigation to deal with statistical techniques, and no attempt was made to work out special procedures for the latent-period data. An approximate method, which employed as the standard error of estimate the over-all average value for the standard deviation of group responses about the calculated regression line, was used in determining the limits of error and in making tests of significance.*

In computing the regression equation the variation in standard deviation with log dose was compensated for by weighting the latent-period observations with respect to the reciprocal of variance (see footnote 5) as well as in accordance with the number of observations.

[7] *The data treated were those of Lettinga* (10).

defect of the mean and a tendency to "tail out" toward the higher latent-period values. A better approximation to the observed frequencies would result, therefore, from use of the empirically established skew curve than from the relationships of a normal curve.

More suitable for present use is the accumulative form of the frequency curve shown in figure 5. The relationships between accumulative frequency and deviate represented by the curve of figure 5 are shown in actual time units (days/30) for the various dose levels by the solid (calculated) lines of figure 1. The transformation to actual time units was accomplished by adding (or subtracting) appropriate multiples of the expected standard deviation to (or from) the expected mean latent period for a given dose. The expected values were derived from the respective regression lines. (See figs. 2 and 3, or equations 1 and 2.) Accumulative percentages corresponding to various multiples of the standard deviation are shown along the ordinate (fig. 5).

The analysis of the frequency distribution was, of necessity, based on the combined data of all dose groups, and the possibility of a variation in degree of

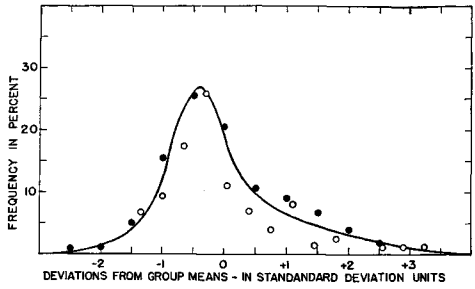

FIGURE 4.—Frequency distribution of latent-period responses obtained with methylcholanthrene, expressed as deviations from group means. The smoothed curve was drawn by sight.

Solid circles: Present data;
Open circles: Data of Shimkin (*11*).

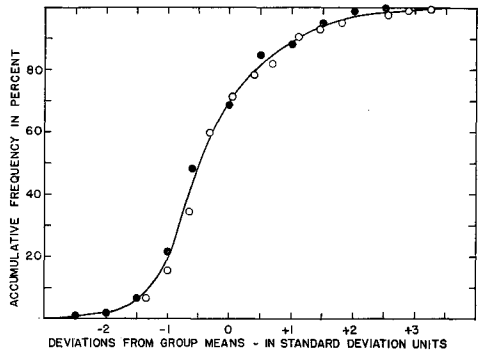

FIGURE 5.—Accumulative frequency curve of latent-period responses obtained with methylcholanthrene, expressed as deviations from group means. The smoothed curve was drawn by sight.

Solid circles: Present data;
Open circles: Data of Shimkin (*11*).

skewness with dose could not be investigated with the limited data available. The above frequency distribution, therefore, represents an over-all average. It has been used to express the results over the entire dose range pending the accumulation of sufficient data for a more detailed analysis. Another question of importance concerns the character of the frequency curve in the dose range where the latent-period response curve is flat, i. e., above 0.5 mg. (−0.301 log mg.). It is possible that the distribution at this dose level will be entirely different from that represented here (see analysis of dibenzanthracene data), but the available data are not sufficient for a separate analysis of this dose range.

Tumor Incidence

The incidence of tumors, in percent, was determined from the "corrected totals" (table 1). The procedures for estimating the corrected totals have been discussed in detail previously (*1*). Briefly, the method is that animals that die without tumors are weighted with respect to the proportion of animals in the tumor popu-

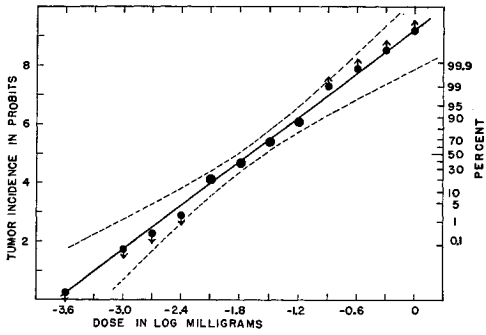

FIGURE 6.—Dose response curve for methylcholanthrene showing the regression of probits on log dose.

Circles: Empirical probits corresponding to observed percentages;

Circles with arrows: Estimated probit values for 0- and 100-percent responses;

Solid line: Calculated regression line;

Curved dash lines: Limits of error of calculated regression line for $P=0.05$.

lation that would be expected to have developed tumors at or prior to the time of death of the animal under consideration.

Tumor incidence in relation to dose.—For a statistical evaluation of the data, the percentage values were converted into probits (table 1), and the doses to logarithms (table 1, log milligrams). The regression of probits on log dose is adequately described by a straight line (deviations of the observed values from a straight line are not statistically significant) thus indicating that the logarithms of individual effective doses of methylcholanthrene are normally distributed. The relations are shown in figure 6.

The regression equation is:

$$Y=5.098+2.474\ (X+1.641) \quad (3)$$

where Y is the estimated probit corresponding to dose X in log milligrams.

The percentage equivalents of the observed and calculated probits are given in figure 7. The S-shaped curve of this figure permits a better visualization of the practical dose limits within which the percentage response may be used for quantitative studies. Without taking into consideration the limits of error of the estimated curve, the useful dose range is seen to be from about 0.0045 mg., the dose calculated to give tumors in 5 percent of the mice (TD_5), to about 0.096 mg., the dose which would be expected to yield a 95-percent incidence of tumors (TD_{95}). Beyond these limits the responses rapidly approach 0 and 100 percent, respectively, and there is no further correlation between dose and tumor incidence.

Median tumor dose (TD_{50}).—The dose of methylcholanthrene which would be expected on a basis of the present data to produce tumors in 50 percent of the test animals is -1.681 ± 0.063 log mg., or $0.021^{+0.0032}_{-0.0028}$ mg. The limits of error corresponding to $P=0.05$ are ±0.123 log mg., or $^{+0.0068}_{-0.0051}$ mg.

Standard deviation of the logarithms of individual effective doses about log TD_{50}.—Gaddum (*12*) has shown that the S-shaped dose-response curve may be considered as an integrated frequency curve of individual effective doses and that the standard de-

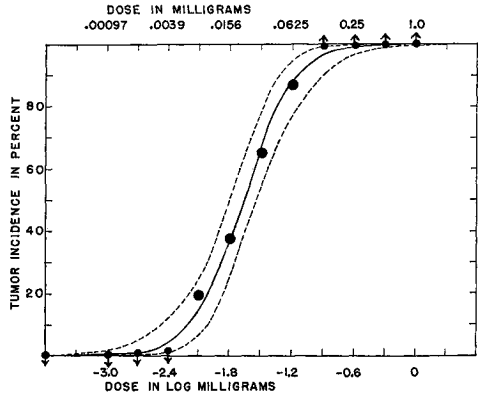

FIGURE 7.—Dose response curve for methylcholanthrene showing relation of tumor incidence in percent to log dose. Transformed from data of figure 6.

viation, λ, of the logarithms of individual effective doses about the median effective dose may be estimated from this curve or from its counterpart in probability units, e. g., probits (*5*), or normal equivalent deviations (*12*). In the instance of methylcholanthrene, the value of λ estimated from probit data is 0.40 ± 0.06. The limits of error corresponding to $P=0.05$ are ± 0.13.

The statistic λ has been widely used in recent years for comparing the biologic actions of various drugs. It has also been used for comparing the responses of different types of test animals to a common material. Thus, Boyland and Warren (*13*) reported λ values of 0.58 and 0.73, respectively, for mice of strains CBA and Simpson which were injected subcutaneously with a lard solution of methylcholanthrene. The present estimate of the standard deviation, 0.40, is less than the values found by Boyland and Warren, but it is still within the limits for "normal drug action" as quoted by these authors, i. e., 0.37 to 0.91. It would appear, then, that the action of tricaprylin solutions of methycholanthrene in C3H male mice is less variable than that of lard solutions in CBA and Simpson strain mice.

Relative weights of tumor-incidence responses at different dose levels.—The reliability of a dosage, or potency, estimate inferred from a percentage (or probit) response varies according to the magnitude of the response itself, as well as with the number of unit observations on which it is based. Weighting coefficients for use with percentage (or probit) responses of various magnitudes have been published by Gaddum (*12, fig. 9*) and by Bliss (*5, table III*). The appropriate values corresponding to the expected percentage responses for methylcholanthrene are given in table 3, column 4. The relative numbers of mice that would be required to give percentage responses of equal weight, with respect to estimates of potency (table 3), are those which would be required if 10 were used at the 50-percent level.

TABLE 3.—*Relative weights of tumor-incidence observations and numbers of mice required for observations of equal weight at various doses of methylcholanthrene*

Dose (in milligrams)	Expected probit	Expected percent	Weighting coefficient [1]	Mice required for equal weight responses
				Number
1.0	9.158	100.0	0.000	
0.5	8.413	99.97	.004	1,667
0.25	7.669	99.62	.032	200
0.125	6.924	97.3	.148	43
0.062	6.179	88.1	.378	17
0.031	5.435	66.8	.594	11
0.0156	4.690	37.9	.614	10
0.0078	3.945	14.6	.420	15
0.0039	3.201	3.6	.180	35
0.00195	2.456	.55	.045	141
0.00098	1.711	.05	.006	1,111
0.000244	.222	0	.000	

[1] From Bliss (*5, table III*).

Joint Use of Latent-Period and Tumor-Incidence Data

Total numbers of injected mice required to give latent-period responses of equal weight at various dose levels.—The numbers of mice with tumors that would be required to produce latent-period responses of equal weight are given in table 2. It has been shown that the frequency of tumors among injected animals decreases progressively with the dose of methylcholanthrene (figs. 6 and 7). In order, therefore, to obtain a desired number of mice with tumors at a given dose level, it would be necessary to increase the total number injected to allow for those that fail to develop tumors. The expected tumor frequencies and the total numbers of injected mice based thereon are also given in table 2. The total numbers (column 8) represent approximations only, since the relations on which they are based are subject to variation. Account has not been taken of mice that die during the course of the experiment.

Specific induction time.—The specific induc-

tion time was previously defined (*1*) as the time required, on an average, for the production of tumors with dose TD_{50}. The value of this measure for comparing the relative rates of action of different carcinogenic hydrocarbons has already been emphasized (*1*). The specific induction time for methylcholanthrene is 5.14 ± 0.55 months (days/30).[8] The limits of error corresponding to $P = 0.05$ are ± 1.05 months (days/30).

Time-tumor frequency relationships at successive dose levels.—Time-frequency curves have been widely used in presenting the results obtained with single doses of carcinogenic hydrocarbons or in comparing the results obtained with two or more preparations of such materials. This procedure gives detailed information regarding the latent periods of individual animals of a group and offers the advantage that the responses to various preparations may be compared on a common basis at any particular time during the course of an experiment. Thus, the essential information of an experiment may be obtained at a time when most of the tumors have appeared, e. g., 9 months, but several months before full opportunity has been allowed for the extreme cases to develop, e. g., 12 to 18 months. On the other hand, quantitative determinations of the potency relationships between various preparations of a carcinogenic agent are simplest when a maximum time has been allowed for tumors to appear and when the estimates are based on final percentage observations, i. e., on observations which are no longer changing with time. Potency relationships may, nevertheless, be established from observations made during the period when tumors are still appearing provided the time-frequency relationship is known for successive levels of potency (or dosage) and a series of standard curves is available for comparison. Such a series of standard curves may be constructed from the latent-period and tumor-incidence relationships presented previously. For example, the percentage of mice in the tumor population which would be expected to develop tumors at, or prior to, a given time is shown for various levels of dosage by the calculated lines of figure 1. These percentages may be converted to percentages of the total injected population, and the time frequency relationship expressed in terms of the total numbers of injected mice in each dose group.

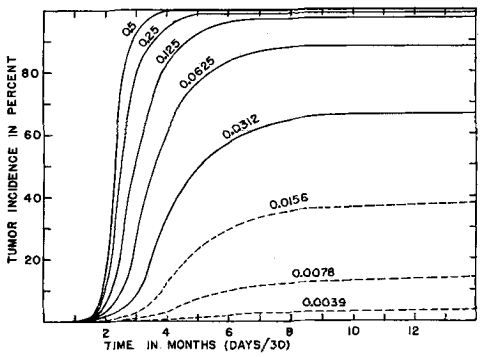

FIGURE 8.—Time-frequency curves for successive doses of methylcholanthrene showing the calculated incidence of tumors at successive periods.

The time-frequency curves for the various doses of methylcholanthrene are shown in figure 8. Although this series of curves illustrates the nature of the time-frequency results to be expected at different levels of dosage and while it may be profitably used in the planning of future experiments under the present conditions, it cannot at present be considered as a standard for quantitative comparison. For the latter usage it would be necessary to prove (1) that this estimate is fairly close to the true results that would be obtained with very

[8] *The value 5.14 was obtained by substituting* -1.681, *log* TD_{50}, *for* X *in equation 2 and solving for* Y, *the expected latent period at this dose level. The limits of error were estimated by taking into consideration the error of estimate of log* TD_{50} *as well as the error of the latent period regression equation.*

large numbers of observations, and (2) that the animal population remains relatively stable over long periods of time. No attempt has been made to set the limits of error of the curves of figure 8, since involved in their estimation are errors of the latent-period response curve, errors of the probit response curve, errors of the standard-deviation regression line, and finally errors of estimate of the latent-period frequency distribution.

In spite of wide possible limits of error of the curves of figure 8, some information concerning their nature has been gained. For example, it is apparent that tumors may occur practically as early with the lower doses as with the higher doses of methylcholanthrene although the probability of early tumors becomes less as the dose is decreased. Furthermore, the curves asymptote at various levels below 100 percent as the dose is decreased below about 0.5 mg., and an incidence of 100 percent would never be reached at these low doses regardless of the length of time the observations are continued.

Dibenzanthracene

The summarized results obtained with dibenzanthracene in doses of 0.00195 to 8.0 mg. are presented in table 4. The discussion concerning analytical procedures in the section on methylcholanthrene is applicable also to dibenzanthracene, and the reader is referred to that section for explanation of the methods of presentation and the purposes of the various analyses.

Table 4.—*Summarized results obtained on latent period of tumors and tumor incidence of mice injected with dibenzanthracene*

Dose [1]		Mice injected	Mice with tumors	Latent period			Tumor incidence			
				Mean latent period [2]	Standard deviation [2]	Weight of observation [3]	"Corrected total" [3]	Mice with tumors	Probits [4]	Weight of observation
Mg.	*Log mg.*	*Number*	*Number*	*Months*	*Months*		*Number*	*Percent*		
8.0	+0.903	21	16	3.75	0.32	16.0	16.0	100.0		
4.0	+.602	20	17	3.83	.62	17.0	18.6	91.5	6.372	
2.0	+.301	19	19	3.69	.64	19.0	19.0	100.0		
1.0	0	22	22	3.60	.54	22.0	22.0	100.0		
0.5	−.301	21	20	3.76	.56	15.4	20.1	99.5		
0.25	−.602	21	19	4.01	1.16	7.11	20.0	95.0	6.645	
0.125	−.903	23	21	4.47	1.48	4.62	21.9	95.9	6.739	4.58
0.062	−1.204	20	20	5.10	1.91	2.90	20.0	100.0	[5] 6.726	8.46
0.031	−1.505	21	16	6.31	1.67	1.63	19.8	80.8	5.870	11.44
0.0156	−1.806	19	6	5.94	1.41	.46	15.8	38.0	4.694	10.05
0.0078	−2.107	40	6	8.78	2.61	.35	32.1	18.7	4.111	17.30
0.00195	−2.709	79	2	9.48	2.40	.08	61.9	3.2	3.148	10.27

[1] The volume of solvent used for each injection was 0.5 cc. at dose 8.0 mg.; 0.25 cc. at all other doses.
[2] Months=Days/30.
[3] See text.
[4] See Bliss (*5*, *7*).
[5] Estimated probity for the 100-percent response.

Latent Period

Standard deviation.—Inspection of figure 9 reveals that above dose 0.5 mg. the variability of individual latent periods is relatively stable whereas below this dose variability increases progressively as the dose is decreased. The standard deviations of individual latent periods obtained with different doses are listed in table 4 and are shown graphically in figure 10. The regression of standard deviation on log dose is probably significant in the range 0.00195 to 0.5 mg., but there is no correlation between standard deviation and

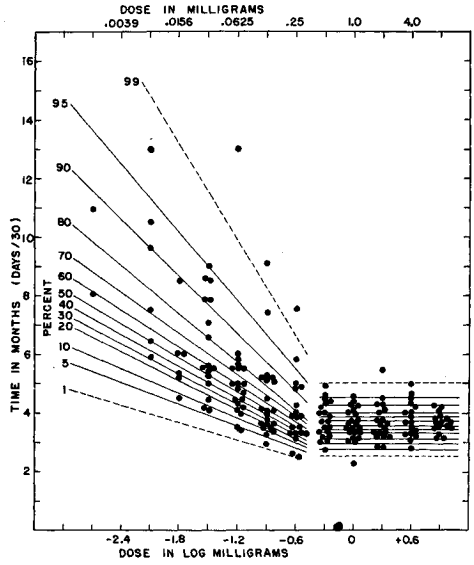

FIGURE 9.—Latent periods of tumors induced with dibenzanthracene. Solid circles represent individual observations plotted to avoid superposition of points at given doses. The oblique and horizontal lines show the calculated variation at continuous dose levels.

dose in the range 1.0 to 8.0 mg. The regression in the lower dose region may be represented as linear as shown by the oblique line (fig. 10). The equation to this line is:

$$\sigma_c = 0.935 - 0.894\ (X + 0.649) \qquad (4)$$

where σ_c represents the calculated value of the standard deviation in months (days/30) and X the dose in log milligrams. It is apparent that at about dose 0.5 mg. (−0.301 log mg.) the standard deviation reaches a minimal level below which it is not further reduced in spite of progressive increases in dosage. On the other hand, with doses of dibenzanthracene smaller than 0.5 mg. the standard deviation becomes progressively greater as the dosage is decreased.

Relative weights of latent-period responses at different dose levels.—The relative weights of latent-period observations when equal numbers of tumorous mice are available for the successive doses of dibenzanthracene are shown in table 5. They represent the relative weights when the maximum weight is considered as unity.

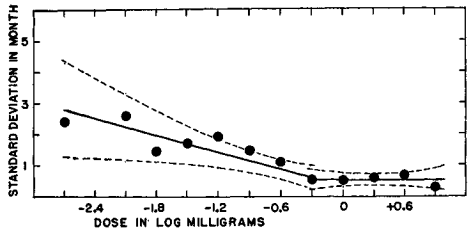

FIGURE 10.—Standard deviation of latent periods with successive doses of dibenzanthracene. Circles: Observed standard deviations; Solid line: Calculated regression line; Curved dash lines: Limits of error of calculated regression line for $P=0.05$

TABLE 5.—*Relative weights of latent-period observations and numbers of mice required for observations of equal weight at various doses of dibenzanthracene*

Dose (in milligrams)	Expected mean latent period [1]	Expected standard deviation [1] (σ_c)	Reciprocal of variance ($1/\sigma_c^2$)	Relative weight (weighting coefficient) [2]	Mice with tumors required for responses of equal weight	Expected incidence of tumors	Total injected mice required for latent period responses of equal weight
	Months	*Months*			*Number*	*Percent*	*Number*
8.0	3.72	0.544	3.38	1.0	10	90 to 100	10 or 11
4.0	3.72	.544	3.38	1.0	10	90 to 100	10 or 11
2.0	3.72	.544	3.38	1.0	10	90 to 100	10 or 11
1.0	3.72	.544	3.38	1.0	10	90 to 100	10 or 11
0.5	3.72	.624	2.57	.760	13	90 to 100	13 or 14
0.25	3.88	.893	1.25	.370	27	90 to 100	27 to 29
0.125	4.61	1.16	.743	.220	45	90 to 100	45 to 48
0.062	5.34	1.43	.489	.145	69	91.2	76
0.031	6.08	1.70	.346	.102	98	74.8	131
0.0156	6.81	1.97	.258	.076	132	49.4	267
0.0078	7.54	2.24	.199	.059	169	24.2	698
0.0039	8.27	2.51	.159	.047	213	8.3	2,566
0.00195	9.00	2.78	.129	.038	263	1.9	

[1] Months=Days/30.

[2] See text.

The relative numbers of mice with tumors required to give latent-period responses of equal weight when 10 are used at the level of maximum weight are shown in table 5, also the total numbers required to give the desired numbers with tumors. (See section entitled "Joint Use of Latent Period and Tumor-Incidence Data.")

Mean latent period.—The mean latent periods for the various doses of dibenzanthracene are listed in table 4, colum 5; their trend with log dose is shown graphically in figure 11. There is a statistically significant regression of mean latent periods on log dose within the dose range 0.00195 to 0.25 but not within the range 0.5 to 8.0 mg. The responses within the latter dose limits undoubtedly represent the minimal limiting level of the latent period for dibenzanthracene under the present condition. The average value for the minimal latent period, represented by the horizontal line (fig. 11) is 3.72 months (days/30) which is identical with that found for dibenzanthracene in previous analyses (*1*) of the data of Lettinga (*10*).

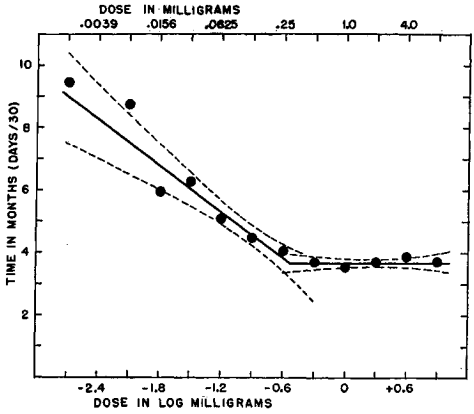

FIGURE 11.—Dose response curve for dibenzanthracene, showing regression of mean latent period on log dose.

Circles: Observed mean latent periods;
Solid line: Calculated regression line;
Curved dash lines: Limits of error of calculated regression line for $P=0.05$.

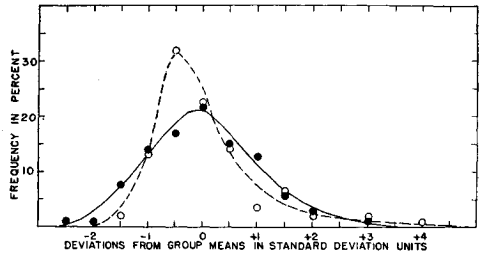

FIGURE 12.—Frequency distributions of latent-period responses obtained with dibenzanthracene, expressed as deviations from group means. The smoothed curves were drawn by sight.

Solid circles: Data of dose groups 0.5 to 8.0 mg.;
Open circles: Data of dose groups 0.00195 to 0.25 mg.

The regression of mean latent periods in the dose range 0.00195 to 0.25 mg. is represented by the oblique line (fig. 11), the equation to which is

$$Y=4.709-2.427\ (X+0.942) \qquad (5)$$

where Y represents the estimated value of the mean latent period in months (days/30) and X the dosage in log milligrams.

Frequency distribution of individual latent-period observations.—In order to obtain sufficient data for an analysis of the frequency distribution, it was necessary to combine the data of the various dose groups. For this purpose the variates were expressed as deviations from group means in standard deviation units. Since it was found that the trend of the mean responses could be divided into two components, the data corresponding to the oblique and horizontal components of the response curve of figure 11 were compiled in separate lots. The frequency distribution curve obtained with the combined data of dose groups 0.00195 to 0.25 mg. is shown by the broken curve of figure 12. The solid curve shows the distribution for dose groups 0.5 to 8.0 mg. The accumulative forms of the frequency curves are shown in like manner in figure 13.

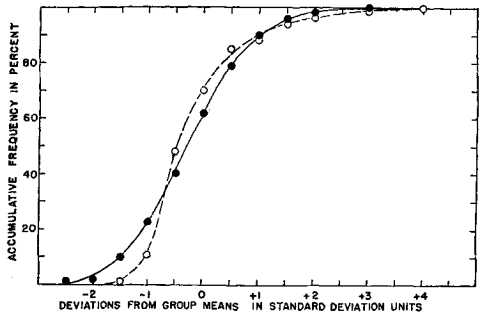

FIGURE 13.—Accumulative frequency curves of latent-period responses obtained with dibenzanthracene, expressed as deviations from group means. The smoothed curves were drawn by sight.

Solid circles: Data of dose groups 0.5 to 8.0 mg.;

Open circles: Data of dose groups 0.00195 to 0.25 mg.

The relations between accumulative frequency in percent, and deviate in standard deviation units, obtained by reference to the smoothed curves of figure 13, were employed in determining the expected accumulative frequency levels indicated by the calculated lines of figure 9. (See discussion of similar determinations with methylcholanthrene.)

Tumor Incidence

The corrected totals and the tumor-incidence responses obtained with dibenzanthracene are given in table 4. The empirical probits corresponding to the percentage estimates are also listed. The response to dose 4.0 mg. (0.6 log mg.) is considerably out of line with the trend of the remainder of the data when plotted in probability units (probits) (fig. 14). A similar phenomenon was noted in an earlier analysis (*1*) of the data of Lettinga (*10*), and, following the same procedure of analysis, this apparent discordant result was omitted in making the first approximation to the trend of the results. The computed regression line (solid line) is steeper than the apparent trend (dash line). The procedure of curve fitting developed by Bliss (7) for application to data on small numbers of animals, or data that are relatively variable about the smoothed trend, involves a series of approximations, using for each new approximation the preceding computed curve for correcting the probit values and their relative weights. In the present analysis a series of such approximations was carried out, and with each new approximation the corrected probit for dose 0.25 mg. (−0.602 log mg.) decreased progressively whereas the corrected values for the other empirical probits became practically identical with the empirical values themselves. Also with each new approximation, the estimated weight of the response to dose 0.25 mg., as estimated from the computed regression line, grew smaller, and the slope of the regression line became steeper, approaching the oblique line shown in figure 15. The low corrected probit for dose 0.25 mg. when considered together with the discordant result previously mentioned for dose 4.0 mg. indicated that the

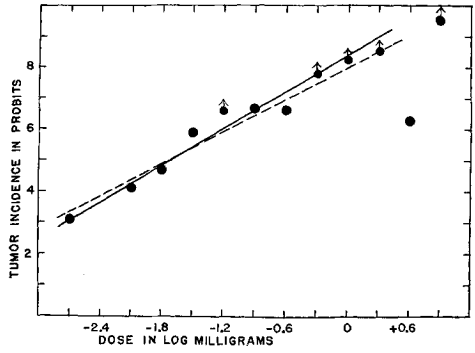

FIGURE 14.—Probit-dose relations obtained with dibenzanthracene.

Circles: Empirical probits corresponding to observed percentages;

Circles with arrows: Estimated probit values corresponding to 100-percent responses;

Dash line: Provisional regression line, drawn by sight;

Solid line: First calculated approximation to regression line.

probit-dose relationship for the higher doses of dibenzanthracene might actually be different from that obtained with lower doses. This was further indicated by the findings of Shimkin and Andervont (*9*) who studied the dose range 0.25 to 3.0 mg. under conditions similar to those of the present experiments. Their results all fell between 90 and 95 percent and were not correlated with dose. Additional evidence of erratic results with higher doses of dibenzanthracene is contained in the results of Lettinga (*10*), previously discussed in this connection (*1*), as well as in some unpublished data of Shear.[9] In view of these findings it seemed advisable to consider 0.125 mg. as the upper limit of the significant dose range and to determine the probit-dose relationship only in the dose region below this level. Accordingly, the

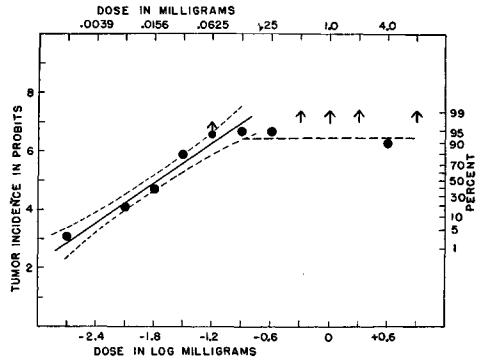

FIGURE 15.—Dose response curve for dibenzanthracene showing the regression of probits on log dose.
Circles: Empirical probits corresponding to observed percentages;
Circle with arrow: Estimated probit value for 100-percent response;
Arrows: Arrows designate doses that gave 100-percent responses in the region where the results were erratic;
Solid line: Calculated regression line for dose range 0.00195 to 0.125 mg.;
Curved dash lines: Limits of error of calculated regression line for $P=0.05$;
Horizontal dash line: Average level of erratic responses in dose region 0.25 to 8.0 mg.

[9] *Personal communication.*

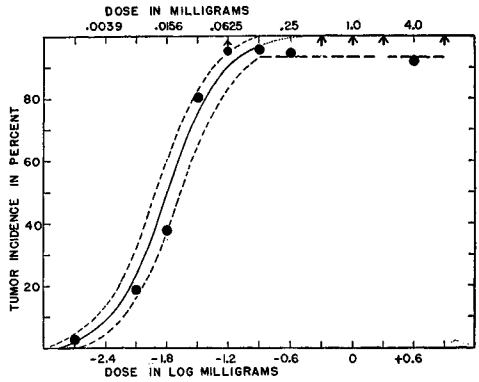

FIGURE 16.—Dose response curve for dibenzanthracene, showing relation of tumor incidence in percent to log dose. Transformed from data of figure 15. The dotted extension of the S-shaped curve shows the relations that would be expected to hold if the responses to the higher doses had followed the same trend as those of the lower doses.

response curve for dibenzanthracene was recalculated by using only the data of dose groups 0.00195 to 0.125 mg. The curve is shown in figure 15. The regression equation is:

$$Y=4.919+2.272\ (X+1.835) \qquad (6)$$

where Y is the expected incidence of tumors in probits and X is the dosage in log milligrams. The relations of figure 15 are shown in terms of percent in figure 16. The horizontal line, indicating a break at about dose 0.25 mg., shows the average level of the erratic responses.

The significant dose range for dibenzanthracene is from about 0.003 mg. (dose TD_5) to about 0.084 mg. (dose TD_{95}). The break in the probit response curve does not alter appreciably the useful dose range.

Median tumor dose (TD_{50}).—The dose of dibenzanthracene that would be expected to produce tumors in 50 percent of the injected animals was estimated from the present data to be -1.799 ± 0.056 log mg. The limits of error of the estimate corresponding to $P=0.05$ are ±0.11. In terms of milligrams, TD_{50} is, therefore,

$0.016^{+0.0022}_{-0.0019}$, and the limits corresponding to $P=0.05$ are $^{+0.0046}_{-0.0035}$.

Standard deviation of logarithms of individual effective doses about log TD_{50}.—The standard deviation, λ, of the logarithms of individual effective doses found from the present studies on dibenzanthracene, is 0.44 ± 0.05. The limits of error for $P=0.05$ are ± 0.094. This value does not differ significantly from tnat for methylcholanthrene in the preceding section, 0.40.

Relative weights of tumor-incidence responses at different dose levels.—Table 6 gives the expected probits and their equivalents in percent, for the various doses of dibenzanthracene. The values were computed from equation 6. The weighting coefficients were determined by reference to the tabled values published by Bliss. They represent the relative weights of the various percentage responses when the number of animals is the same in each dose group. The relative numbers of mice required to give responses of equal weight when 10 are used at the level of the 50-percent response (at dose TD_{50}) are presented in the last column of table 6.

Joint Use of Latent-Period and Tumor-Incidence Data

Total numbers of injected mice required to give latent-period responses of equal weight at various dose levels.—The relative numbers of mice with tumors required for observations of equal weight have been discussed in a preceding section. They are listed in table 5. The decreasing incidence of tumor-bearing mice with successively smaller doses of dibenzanthracene is shown in column 7 of table 5. The values represented are the expected percentages derived from the dose-response relationships developed (figs. 15 and 16). The last column shows the total numbers of mice that would have to be injected in order to obtain the desired numbers with tumors indicated by column 6.

TABLE *6.—Relative weights of tumor-incidence observations and numbers of mice required for observations of equal weight at various doses of dibenzanthracene*

Dose (in milligrams)	Expected probit	Expected percent	Weighting coefficient	Mice required for equal weight responses
				Number
8.0 to 0.125	6.282 or greater.	90 to 100		
0.0625	6.353	91.2	0.318	20
0.0312	5.669	74.8	.540	12
0.0156	4.985	49.4	.636	10
0.00781	4.301	24.2	.532	12
0.00390	3.617	8.3	.302	21
0.00195	2.933	1.9	.117	54

Specific induction time.—The specific induction time is 6.79 ± 0.45 months (days/30). The limits of error corresponding to $P=0.05$ are ± 1.06.

The value 6.79 is identical with a previous estimate (*1*) of the specific induction time made from Lettinga's data on dibenzanthracene (*10*).

Time-tumor frequency relationships at successive dose levels.—The time-frequency curves for various doses of dibenzanthracene are shown in figure 17. They were derived, as described in the section on methylcholanthrene, by joint use of the calculated regression lines for both the latent-period and tumor-incidence types

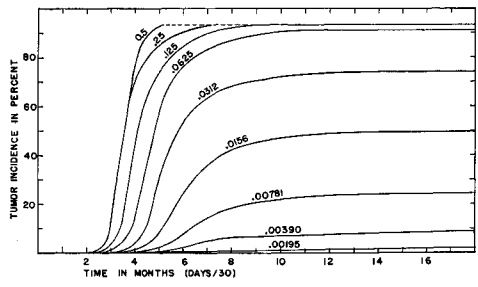

FIGURE 17.—Time frequency curves for successive doses of dibenzanthracene showing the calculated incidence of tumors of successive periods.

of responses as well as the estimated accumulative frequency curves of figure 13. As pointed out in the previous section, the curves are presented as rough approximations only, but they may serve as a guide in the planning of future experiments under these same conditions.

BENZPYRENE

The methods of presentation and the purposes of the analyses of benzpyrene data are similar to those in the preceding sections on methylcholanthrene and dibenzanthracene. Table 7 shows the summarized results obtained with doses ranging from 0.00195 to 8.0 mg. of the hydrocarbon.

Latent Period

Standard deviation.—As in the case of the other hydrocarbons, the results with benzpyrene show a statistically significant correlation between dose and the standard deviation of individual latent periods at all doses below a certain level, but a lack of correlation in the higher dose range. The spread of the individual latent-period observations is illustrated in figure 18. The standard deviations are shown in table 7 and their regression on log dose in figure 19. The relatively low value observed for dose 0.00195 mg. (−2.709 log mg.), was based on only two tumors and hence does not represent a satisfactory estimate. The extension

TABLE 7.—*Summarized results obtained on latent period of tumors and tumor incidence of mice injected with benzpyrene*

Dose [1]		Mice injected	Mice with tumors	Latent period			Tumor incidence			
				Mean latent period [2]	Standard deviation [2]	Weight of observation [3]	"Corrected total" [3]	Incidence of tumors	Probits [4]	Weight of observation
Mg.	*Log mg.*	*Number*	*Number*	*Months*	*Months*		*Number*	*Percent*		
8.0	+0.903	21	20	2.96	0.34	20	20.1	99.5	[5] 8.558	0.141
4.0	+ .602	19	16	3.02	.28	16	16.0	100.0	[5] 8.084	.432
2.0	+ .301	19	19	3.09	.39	14.33	19.0	100.0	[5] 7.626	1.615
1.0	0	20	18	3.32	.62	5.38	19.0	94.7	6.616	3.838
0.5	− .301	19	19	3.86	.83	3.02	19.0	100.0	[5] 6.796	7.011
0.25	− .602	21	14	4.41	1.15	1.39	21.0	66.7	5.432	11.235
0.125	− .903	19	15	5.11	1.49	1.00	18.0	83.3	5.966	11.322
0.062	−1.204	20	4	5.79	1.80	.20	19.8	20.2	4.165	12.038
0.031	−1.505	16	0				15.2	0	[5] 3.428	7.326
0.0156	−1.806	19	0				17.1	0	[5] 3.073	5.250
0.0078	−2.107	40	0				34.4	0	[5] 2.660	5.298
0.00195	−2.709	81	2	8.37	1.32	[6] .03	69.6	2.87	3.102	1.183

[1] Volume of solvent used for each injection was 0.25 cc. at all doses.
[2] Months=Days/30.
[3] See text.
[4] See Bliss (*5, 7*).
[5] Estimated probits for 0- and 100-percent responses.
[6] Provisional value.

of the regression line to this low dose level must therefore be considered as provisional. The extension is shown in figure 19 as a broken line. The regression equation is:

$$\sigma_c = 0.584 - 0.771\ (X - 0.056) \qquad (7)$$

where σ_c is the estimated standard deviation in days/30 and X the dose in log milligrams.

The minimal standard deviation was reached at about 2.0 mg. (0.3 log mg.). The average minimal standard deviation was 0.343 months (days/30).

Relative weights of latent-period observations at different dose levels.—The relative weights of latent-period observations with benzpyrene represent the relative weights when the maximum weight is considered as unity and when the number of mice with tumors is the same at all dose levels. The data are given in table 8.

TABLE 8.—*Relative weights of latent-period observations and numbers of mice required for observations of equal weight at various doses of benzpyrene*

Dose (in milligrams)	Expected mean latent period [1]	Expected standard deviation [1] (σ_e)	Reciprocal of variance ($1/\sigma_e^2$)	Relative weight (weighting coefficient) [2]	Mice with tumors required for responses of equal weight	Expected incidence of tumors	Total injected mice required for latent period responses of equal weight
	Months	*Months*			*Number*	*Percent*	*Number*
8.0	3.02	0.343	8.50	1.0	10	100.0	10
4.0	3.02	.343	8.50	1.0	10	99.7	10
2.0	3.02	.395	6.41	.754	13	98.8	13
1.0	3.30	.627	2.54	.299	33	96.0	34
0.5	3.89	.859	1.35	.159	63	88.8	71
0.25	4.47	1.09	.840	.099	101	75.6	134
0.125	5.05	1.32	.571	.067	149	56.5	264
0.062	5.63	1.56	.413	.049	204	35.8	570
0.031	[2] 6.21	[2] 1.79	[2].313	[2].037	[2] 270	18.6	[2] 1,452
0.0156	[2] 6.79	[2] 2.02	[2].245	[2].029	[2] 345	7.8	----
0.0078	[2] 7.37	[2] 2.25	[2].197	[2].023	[2] 435	.7	----
0.0039	[2] 7.95	[2] 2.48	[2].162	[2].019	[2] 526	.1	----
0.00195	[2] 8.54	[2] 2.72	[2].135	[2].016	[2] 625	0	----

[1] Months=Days/30.

[2] Provisional values.

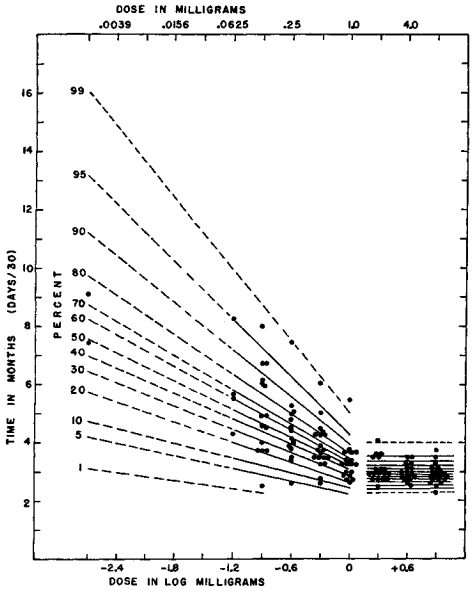

FIGURE 18.—Latent periods of tumors induced with benzpyrene. The solid circles represent individual observations plotted to avoid superposition of points at given doses. The oblique and horizontal lines show the calculated variation at continuous dose levels.

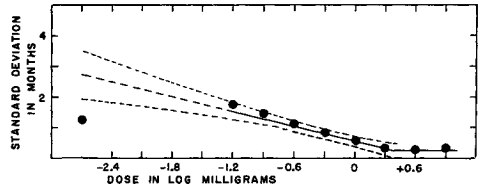

FIGURE 19.—Standard deviation of latent periods with successive doses of benzpy
Circles: Observed standard deviations;
Solid line: Calculated regression line;
Curved dash lines: Limits of error of calculated regression line for $P=0.05$.

The relative numbers of mice with tumors required to give latent-period responses of equal weight are those which pertain when 10 are employed at the level of maximum weight. The total numbers of injected mice required to give the desired numbers with tumors (table 8) are discussed later in the section, Joint Use of Latent-Period and Tumor-Incidence Data.

Mean latent period.—In the dose region below 2.0 mg. the mean latent period increased significantly as the dose was decreased. The observed results gave an excellent fit to a straight line when plotted against the logarithm of dose (fig. 20). The mean latent period for dose 0.00195 mg. was based on only two unit observations and therefore does not represent a reliable estimate. It is fortuitous that it falls so close to the computed regression line which has been extended as a broken

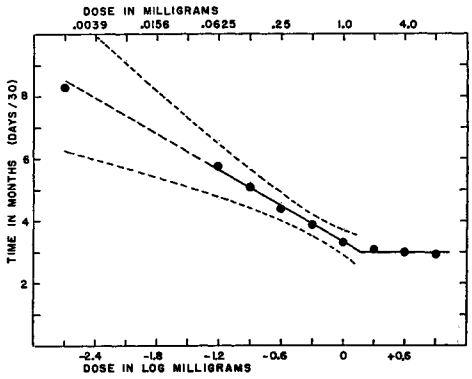

FIGURE 20.—Dose response curve for benzpyrene, showing regression of mean latent period on log dose.

Circles: Observed mean latent periods;
Solid line: Calculated regression line;
Curved dash lines: Limits of error of calculated regression line for $P=0.05$.

line to the low dose region.[10] The regression equation is:

$$Y=3.826-1.931\ (X+0.270) \quad (8)$$

where Y represents the calculated value of the mean latent period in months (days/30) and X the dose in log milligrams.

As already noted, the mean latent period was not correlated with dose in the region 2.0 to 8.0 mg. This is shown by the break in the plotted results in figures 18 and 20. The average value of the minimal latent period was 3.02 months (days/30). It is shown graphically by the horizontal component of the response curve of figure 20.

Frequency distribution of individual latent-period observations.—As in the case of methylcholanthrene, the frequency distribution of individual latent periods was

[10] *The standard deviation of the mean latent periods about the calculated regression line (the error of estimate used for purposes of statistical analysis (see footnote 6)) was much less for benzpyrene than for the other hydrocarbons. That this result is fortuitous is indicated by the fact that figures 1, 9, and 18 show a similar degree of scatter of the individual latent periods. It was decided, therefore, to use as the error of estimate for the benzpyrene data the average of the values found for all three materials rather than the lower value calculated from the benzpyrene results alone.*

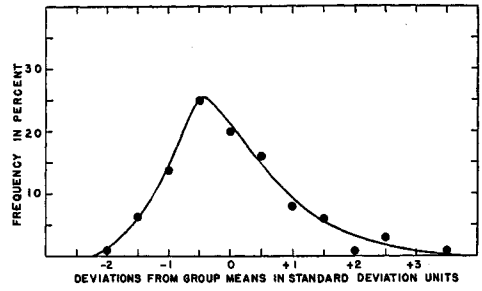

FIGURE 21.—Frequency distribution of latent-period responses obtained with benzpyrene, expressed as deviations from group means. The circles represent observed frequencies. The smoothed curve was drawn by sight.

determined from the combined data of all dose groups. Sufficient data were not available for a separate analysis of the dose groups corresponding to the horizontal component of the response curve. As in the previous analyses, combination of the data was made possible by conversion of the individual variates to deviations from group means in standard deviation units.

The frequency distribution based on the combined data is shown in figure 21, and the accumulative frequency curve in figure 22. The curve of figure 22 was used (as described in section on methylcholanthrene) for deriving the calculated lines of figure 18.

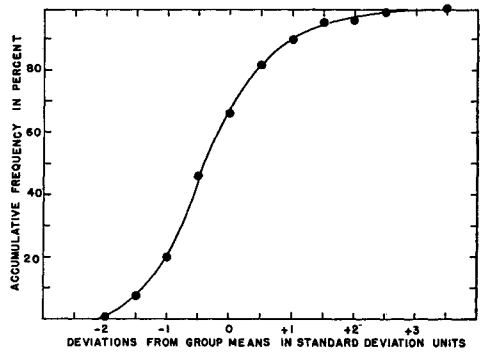

FIGURE 22.—Accumulative frequency curve of latent-period responses obtained with benzpyrene, expressed as deviations from group means. The circles represent observed frequencies. The smoothed curve was drawn by sight.

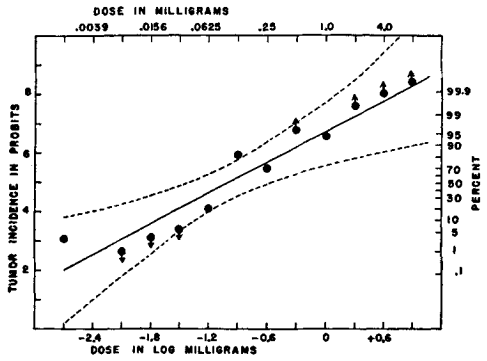

FIGURE 23.—Dose response curve for benzpyrene showing the regression of probits on log dose.

Circles: Empirical probits corresponding to observed percentages;
Circles with arrows: Estimated probit values for 0– and 100– percent responses;
Solid line: Calculated regression line;
Curved dash lines: Limits of error of calculated regression line for $P=0.05$.

Tumor Incidence

The summarized tumor-incidence results are presented in table 7. The observed percentages were converted to probits for the purpose of statistical analysis. The probit values given in table 7 are graphically represented by the solid circles of figure 23. The solid line of figure 23 represents the third computed approximation derived by the method of Bliss (7). It will be noted that two of the plotted points deviate considerably from the trend of the remainder of the data. These deviations are statistically significant, and the plotted points cannot be said to fall consistently about the best straight line drawn through them. Since there is no apparent break or curvature in the trend of the results but rather a wide variation of the plotted points about any single line that can be drawn, the conclusion is that there is a significant degree of heterogeneity in the animal responses. Such results are not infrequently encountered in dose-response investigations with drugs, and methods have been devised (*5, 6, 7*) for their statistical analysis. Whether the present results are characteristic of benzpyrene or whether they are fortuitous and are to be expected with methylcholanthrene and dibenzanthracene as well cannot be said at the present time. It is possible, however, that larger numbers of animals in the various dose groups would yield more consistent percentage responses.

The heterogeneity of the benzpyrene data makes it impossible to draw definite conclusions regarding the true nature of the dose response curve for this material and renders useless certain of the analyses that were made of the other hydrocarbons. However, other useful information may be obtained from the benzpyrene results, and the relation between dose and response may be determined within broad limits of error.

The curved lines of figure 23 show the limits of error of the calculated regression line corresponding to $P=0.05$. They were determined by methods applicable to the analysis of heterogeneous data (7). The equation to the regression line is:

$$Y=4.971+1.753\ (X+1.013) \qquad (9)$$

where Y is the calculated probit value and X the dose in log milligrams.

The percentage equivalents of the probit results are shown in figure 24. As judged from the S-shaped percentage curve without considering the limits of possible error, the useful dose range for benzpyrene is from about 0.012 mg. (TD_5) to about 0.875 mg. (TD_{95}).

Median tumor dose (TD_{50}).—Although subject to relatively large errors of estimate, TD_{50} can be determined within known limits. By appropriate procedures (7) log TD_{50} is found to be -0.996 ± 0.16. The limits of error corresponding to $P=0.05$ are $\pm$ 0.37. Thus, TD_{50} is

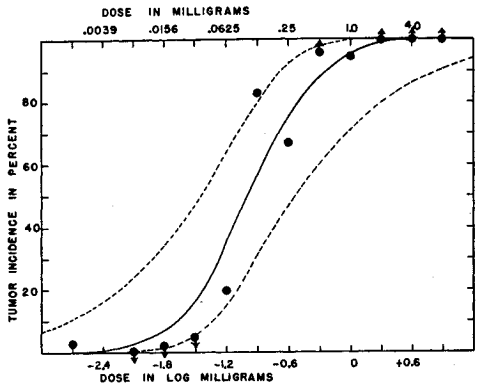

FIGURE 24.—Dose response curve for benzpyrene showing relation of tumor incidence in percent to log dose. Transformed from data of figure 23.

$0.101^{+0.046}_{-0.032}$ mg., and the limits of error for P=0.05 are $^{+0.135}_{-0.058}$.

Joint Use of Latent-Period and Tumor-Incidence Data

Total number of injected mice required to give latent-period responses of equal weight at various dose levels.—The total number of mice that must be injected to give the desired number of tumors (table 8) will depend upon the expected incidence of tumors at each dose level. Although the curve of expected percentages in figure 24 cannot be considered as being very accurately established, it may be used as an approximation in the absence of better information for judging the numbers of animals to be used in future experiments involving the latent period type of response. The expected percentages are given in table 8, as well as the total numbers of mice required for observations of equal weight when 10 are used at the level of maximum weight.

Specific induction time.—The specific induction time for benzpyrene is 5.23 ± 0.59 months (days/30). The limits of error corresponding to P=0.05 are ± 1.51 months.

COMPARISON OF RESULTS OBTAINED WITH THE THREE HYDROCARBONS

Tumor Incidence

The probit-dose response curves for methylcholanthrene, dibenzanthracene, and benzpyrene are plotted in figure 25. The curves for methylcholanthrene and dibenzanthracene (disregarding in the latter instance the higher dose range within which response is not correlated with dose) are fairly close together on the common dose scale, and the slopes of the two curves are essentially parallel. The curve for benzpyrene, however, differs from the others in both position and slope. Analyses were made to determine whether these differences were significant or whether they could have occurred by chance under the condition of the experiment.

Comparison of tumor-incidence response curves.—Statistical analyses show that the differences in slope are not significant as compared with their errors of estimate. This fact indicates that variations in slope of the magnitudes observed could have been due to chance under present condi-

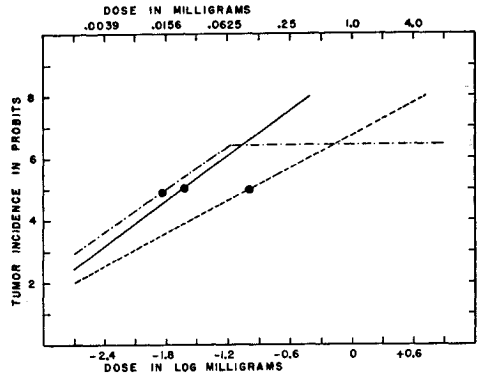

FIGURE 25.—Calculated probit-dose response curves for three carcinogenic hydrocarbons showing their relative positions on an absolute dose scale.
Solid line: Methylcholanthrene;
Dash line: Benzpyrene;
Dash-dot line: Dibenzanthracene;
Circles on lines: Means of experimental observations.

tions. Caution should be exercised, however, in considering the slope to be actually the same for the three materials since the errors of the estimates are relatively large, particularly in the instance of benzpyrene, and it is possible that larger numbers of observations might show significant differences not detectable under present conditions.

In spite of the wide range of error associated with the benzpyrene data, the response curve obtained with this material differs significantly, with respect to position, from those of the other two compounds which do not differ significantly from each other.

Estimation of the relative carcinogenic potencies of the three hydrocarbons.—The term "potency" as used here refers simply to the relative quantities of the various hydrocarbons required to produce a common biologic response. It is recognized that many factors would have to be considered in an attempt to estimate the absolute potency relationship, e. g., differential solubilities, relative rates of elimination from the body, relative rates of detoxification, etc. The potency ratios given represent, therefore, the apparent potencies of the respective hydrocarbons under the prescribed conditions of the experiment.

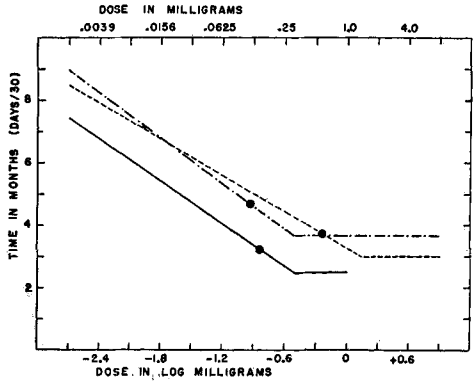

FIGURE 26.—Calculated latent period-dose response curves for three carcinogenic hydrocarbons showing their respective positions on an absolute dose scale.

Solid: Methylcholanthrene;
Dash: Benzpyrene;
Dash-dot: Dibenzanthracene.

The logarithms of the potency ratios were derived by obtaining the differences between the respective values for log TD_{50}. The antilogs of the differences represent the potency ratio, which is 6.3 : 4.8 : 1, in the order dibenzanthracene : methylcholanthrene : benzpyrene.[11] In terms of relative quantities required to produce the same biologic response (50-percent tumors), the ratio is 0.16 : 0.21 : 1. The difference between the relative potencies of methylcholanthrene and dibenzanthracene is not statistically significant, but both of these materials differ significantly from benzpyrene.

The magnitude of this difference in potency, however, cannot be stated with certainty in view of the wide limits of error associated with the results with benzpyrene. The values given represent the best estimates derived from present data. It has not been established that they represent exact values for the true potency ratios.

Latent Period

Comparison on an absolute dose scale (dose in milligrams).—The latent-period response curves for the three hydrocarbons are plotted in figure 26. The curves for methylcholanthrene and dibenzanthracene are essentially parallel, while that for benzpyrene has a somewhat flatter slope.

[11] *Shimkin* (11) *suggested the use of molecular weights rather than absolute weights for comparing relative potencies, "especially if the compared chemicals differ considerably in their molecular weights." The materials used in the present investigation do not differ appreciably in molecular weights. When their potencies are compared on such a basis, the ratio is 6.8: 5.2 : 1, in the order dibenzanthracene (molecular weight 268) : methylcholanthrene (molecular weight 268) : benzpyrene (molecular weight 252).*

Whereas the probit curves (fig. 25) for methylcholanthrene and dibenzanthracene are close together, the latent-period curves for these materials (fig. 26) are widely separated. The benzpyrene curve of figure 26 is nearest the dibenzanthracene curve which it crosses in the oblique, and again in the horizontal segment.

The horizontal portions of the curves in the range where response does not vary with dose are excluded from this and the following analysis. Tests of statistical significance show that the variations in slope are no greater than could have occurred due to chance alone under the conditions of the experiment. (See discussion of similar findings with respect to the probit curves on p. 524.)

The benzpyrene and dibenzanthracene response curves do not differ significantly from each other in relative position, but they both differ significantly from the curve for methylcholanthrene.

Estimation of the relative speeds of action of the three hydrocarbons.—It has already been pointed out (*1*) that latent-period responses are unsatisfactory for comparing the relative carcinogenic potencies of different hydrocarbons [12] since there is no point on the latent-period response curve which is definitely known to have a common significance for different compounds. Nevertheless, it is of value to know the relative speeds of action of different carcinogenic hydrocarbons when they are administered under similar conditions to similar test animals.

The curves of figure 26 indicate that on an average given quantities of dibenzanthracene and benzpyrene require considerably longer periods of time for the production of tumors than do similar quantities of methylcholanthrene. This is true when the comparison is made on a basis of equal quantities, e. g., milligrams, of the respective materials. Equal quantities of the hydrocarbons, however, did not have the same carcinogenic potency as measured by the tumor-incidence response; benzpyrene acted as if it were significantly less potent than the other materials.

If the latent-period data are considered alone, there is no point on the abscissa (dose) scale which could be chosen in preference to all others for comparing the relative speeds of action, i. e., the respective latent-period responses, and satisfactory comparison could be made only if the various response curves were parallel. Since the difference between the slopes was not found to be significant in the present instance, the curves were compared on a basis of a common slope (the compound slope). If the differences in slope should be found upon further experimentation to represent real differences, the present conclusions regarding speeds of action will not be strictly respresentative, since the relative speeds of action will vary at different levels of dosage.

The average differences between speeds of action when the materials were considered in terms of equal quantities were 1.22 months (days/30) for methylcholanthrene and dibenzanthracene, 1.86 months for methylcholanthrene and benzpyrene, and 0.64 months for dibenzanthracene and benzpyrene. The materials in order of rapidity of action were methylcholanthrene, dibenzanthracene, and benzpyrene. It is obvious from figure 26 that this order of relative speeds of action applies only to the oblique components of the respective curves.

Although the crossing of the oblique segments of the benzpyrene and dibenzanthracene curves could be fortuitous, there is little doubt that the horizontal segment of the dibenzanthracene curve crosses the

[12] *The latent-period response may, however, be used for determining relative potencies of different preparation of a given hydrocarbon.*

oblique segment of the benzpyrene curve since the horizontal segment of the latter is significantly lower than that of the former. The relative speeds of action of these two materials are therefore reversed when the higher doses are used.

In view of these findings, it would seem undesirable to make single dose comparisons between benzpyrene and dibenzanthracene with respect to the mean latent period. The comparison would have to be made over a wide range of doses since there is one dose, and possibly two, at which the mean latent period will be exactly the same for the two materials. At other doses the latent period for one material may be greater or less than for the other, depending upon the magnitude of the dose employed.

Comparison on a relative dose scale (dose in carcinogenic units).—Since the quantity of hydrocarbon composing one carcinogenic unit (*1*) was found to differ among the various materials, it would be of interest to know their relative rates of action when quantities possessing the same number of carcinogenic units are compared. Considering dose TD_{50} as one carcinogenic unit in each instance, the respective dose scales were converted to carcinogenic units by expressing the actual quantities of hydrocarbon as fractions or multiples of TD_{50}.

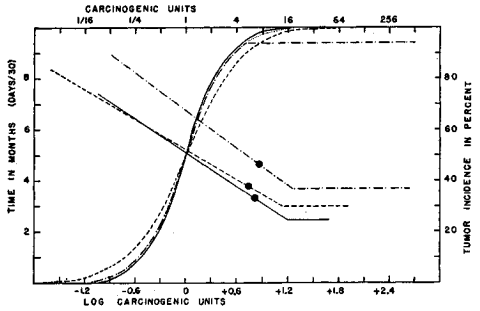

FIGURE 27.—Calculated response curves for three carcinogenic hydrocarbons showing incidence of tumors in percent and latent periods in months on a relative dose scale (carcinogenic units).
S-shaped curves: Incidence data;
Linear curves: Latent-period data;
Solid: Methylcholanthrene;
Dash: Benzpyrene;
Dash-dot: Dibenzanthracene.

The percentage and latent-period response curves for the three hydrocarbons are plotted in figure 27. The lower abscissa scale represents log carcinogenic units, the upper abscissa scale, the relative doses in carcinogenic units. It will be noted that the 50-percent points on the percentage response curves are superimposed at one carcinogenic unit. The respective points on the latent-period curves corresponding to this same dosage represent the specific induction times referred to.

When compared on a basis of similar tumor producing doses, the latent-period curve for benzpyrene falls much closer to the methylcholanthrene curve than to the one for dibenzanthracene (fig. 27). This is in contrast with the findings when absolute dosages were employed (fig. 26). The slopes of the respective response curves were not altered by conversion of the dosages to carcinogenic units. The positions were merely changed so that the respective values of TD_{50} coincided.

The analysis of slopes presented in the preceding section is applicable to the present series of curves. It may be reiterated, therefore, that the slopes were not shown to be significantly different.

When measured with respect to average position for each curve and on the basis of a common slope, the oblique components of the response curves for benzpyrene and methylcholanthrene do not differ significantly from each other. Both of these curves, however, differ significantly from that for dibenzanthracene. The horizontal components of the respective response curves differ significantly among them-

selves, that for benzpyrene being intermediate to the others in position.

Estimation of the relative speeds of action of equal carcinogenic units of the three hydrocarbons.—Comparison of the oblique segments of the curves of figure 27 with respect to their average positions and on the basis of a common slope indicates that tumors produced with dibenzanthracene appeared on an average 1.49 months later than with methylcholanthrene, and 1.22 months later than with benzpyrene. Tumors induced with benzpyrene appeared 0.27 months later than did those induced with methylcholanthrene.

With doses greater than 16 carcinogenic units (1.2 log carcinogenic units), the latent-period response of all materials was minimal. In the region of the minimal responses tumors were produced with dibenzanthracene 1.24 months later than with methylcholanthrene, and 0.7 months later than with benzpyrene. The tumors induced with benzpyrene appeared 0.54 months later than did those induced with methylcholanthrene.

If response curves are not parallel, the differences in respective speeds of action will vary at different levels of dosage, and as already pointed out it would be difficult to select a point of reference on the basis of latent-period data alone. In conjunction with the percentage data, however, it would be possible to compare relative speeds of action at doses giving a common percentage response and thus to have points of reference which possess a common significance in the instance of different hydrocarbons. The use of dose TD_{50} as a point of reference for comparing relative speeds of action has already been described (specific induction time (*1*)). If the present results are compared on a basis of their specific induction times, methylcholanthrene and benzpyrene differed by only 0.09 months in their respective speeds of action while the speed of action of dibenzanthracene was slower by 1.65 and 1.56 months, respectively, when compared with the other materials. These results are in contrast with the differences between minimal latent periods noted previously.

DISCUSSION

One of the primary purposes of this study was to supply additional data on the nature of the relationship between dose of subcutaneously injected carcinogenic hydrocarbons and the local tumor responses which they elicit in test mice. It is necessary to know the nature of the dose-response relationship in order that appropriate statistical procedures may be employed in analyses of the biologic results and in testing the significance of differences obtained under various experimental conditions. In a previous report (*1*) it was pointed out that statistical analysis of dose-response data is simplest when the relationship between dose and response is linear and that statistical methods are available for the analysis of data that follow this type of relationship. Attempts were made (*1*) to find appropriate units for expressing both dose and response so that the relationship between them would be linear. It was found that certain units which were applicable in the instance of most drugs were also applicable in the instance of carcinogenic hydrocarbons.

The findings of the previous study are substantiated by the present results. They are summarized as follows: (1) The mean latent period of tumors appearing in a group of mice, all of which have received the same dose of hydrocarbon, bears a linear relationship to the logarithm of the dose. This relationship is limited, however, owing to the fact that there is a minimal time below which tumors do not appear with further increases in dose. The minimal latent period appears to be

characteristic for a given hydrocarbon under constant experimental conditions. (2) The tumor-incidence responses, in percent, follow a symmetrical S-shaped curve possessing the characteristics of an integrated normal curve when plotted against logarithm of dose. Such S-shaped curves may be converted to a straight line by transforming the percentages to probability units (probits (*5*), or normal equivalent deviation (*12*)) and plotting them against the logarithm of dose.

The conclusions regarding the mean latent period-dose relationship are substantiated by the results with all three of the compounds employed. Additional evidence of a linear relationship between the mean latent period and the logarithm of dose was obtained by Gottschalk (*15*) in studies with benzpyrene.

The percentage-dose relationship was confirmed by the results obtained with methylcholanthrene and dibenzanthracene, but the data obtained with benzpyrene proved to be heterogeneous and not strictly definable by the relations of a normal curve. It is possible, however, that percentage responses to benzpyrene based on larger numbers of mice will give more homogeneous results. (See data of Leiter and Shear (*16*).)

A second objective of this study was to compare the relative carcinogenic potencies and the relative speeds of action of methylcholanthrene, dibenzanthracene, and benzpyrene. Attempts were not made to estimate absolute potencies but rather the relative actions, or the apparent potencies, of the three materials when administered under identical test conditions. For the conditions of the present experiments, the carcinogenic potencies were in the order dibenzanthracene, methylcholanthrene, benzpyrene; the ratio was 6.3:4.8:1. The potency difference between dibenzanthracene and methylcholanthrene was not statistically significant and the two may be exactly the same, or either may be slightly more potent than the other to a degree not detectable in the present experiment. In any event, it would not be surprising if the order were reversed in a subsequent experiment under the same conditions. There seems to be no doubt, however, that larger quantities of benzpyrene were required to produce a 50-percent response than of either dibenzanthracene or methylcholanthrene and that the apparent potency of benzpyrene is comparatively less under the conditions of these experiments. The order of potency here is the same as that found by Andervont and Shimkin (*17*) with regard to the induction of pulmonary tumors. Their results showed that dibenzanthracene produced the greatest number of pulmonary tumors, with methylcholanthrene next in potency. Benzpyrene produced only one-tenth as many pulmonary tumors as did methylcholanthrene and only one-sixteenth as many as dibenzanthracene when the materials were injected subcutaneously in equal quantities (*17*, *table 3*). This order of relative potencies of the three compounds is apparently contradictory to that usually given in the literature for the materials when subcutaneously injected. However, it should be pointed out that, to our knowledge, no previous comparative studies have been made in which the lower doses were used. Under the conditions of the present experiments doses of dibenzanthracene greater than about 0.1 mg. might be expected to give aberrant percentage results which, in the absence of a complete dose response curve, might be interpreted as indicating a lower carcinogenic potency for dibenzanthracene (fig. 25).

The most rapidly acting of the three compounds was methylcholanthrene, irrespective of whether comparisons were

made on an absolute dose basis (in terms of milligrams) or in terms of equal tumor-inducing doses (carcinogenic units). By using the latter comparison, the speeds of action were in the order methylcholanthrene, benzpyrene, dibenzanthracene. When comparisons were made on an absolute dose basis, the conclusions regarding the respective speeds of action of dibenzanthracene and benzpyrene depended on the dose level at which the comparisons were made.

In addition to the primary objectives of the experiments other useful information was obtained. For example, with all three hydrocarbons there was a significant correlation between the standard deviation of individual latent periods and the logarithm of dose. The range of variation of individual latent periods was strikingly large, especially in the region of the lower doses (figs. 1, 9, and 18). In the present experiments the various dose groups were made up by the addition of animals over a period of about 3 months, and it is possible that a wider range has been observed under these conditions than would have been obtained if the sampling had been made from a single lot of animals. The present results are probably more representative, however, of the variations to be expected in the population as a whole. The increasing range of variation of the latent period as the dose is decreased, coupled with the coincident progressive decrease in the percentage of mice with tumors, limits the usefulness of the latent-period response in quantitative studies to a very narrow dose range. For example, above the maximum effective dose, that which just produces the minimal latent period (*14*), there is no correlation between dose and response, and the latent period cannot be used as a criterion of quantitative biologic activity. On the other hand doses smaller than TD_{50} produce tumors in less than half of the injected mice, and the range of variation in the latent periods is so great that the numbers of mice required for reliable group responses are too large to be practical. The dose range within which the latent period might be effectively used is therefore from about dose TD_{50} to the maximum effective dose.

Another point of interest is the apparent common significance of the maximum effective dose of all three hydrocarbons. The maximum effective dose was defined by Fieser (*14*) as the dose that just produces the minimal latent-period response. Fieser noted that at this dosage the percentage response "approaches 100 percent." In previous work (*1*), the present authors questioned the use of the maximum effective dose for comparing the relative potencies of different carcinogenic agents because of the lack of proof that this criterion possessed the same significance, with respect to potency, for different agents. However, the present findings indicate that it does have a common significance, at least for the three hydrocarbons used. Reference to figure 27 reveals that the dose opposite the point of junction of the oblique and horizontal components of the latent-period response curve (the maximum effective dose) corresponds to the point on the corresponding percentage curve at which, for practical purposes, the latter just reaches 100 percent. Actually the approach to 100 percent is asymptotic, and the expression "just reaches 100 percent" is theoretically meaningless. However, as Foster (*18*) points out, the dose which gives a 99-percent response may for practical purposes be considered as that which just produces 100 percent.

The percentages corresponding to the maximum effective doses were calculated

from the probit regression equations to be 99.8 and 97.8 for methylcholanthrene, and benzpyrene, respectively. The dibenzanthracene percentage response curve did not continue to 100 percent (fig. 16), but the results became erratic with doses greater than 0.125 mg. If the trend of the responses at the lower doses is continued, however (see dotted extension of dibenzanthracene percentage curves of figures 16 and 27), the percentage response corresponding to the maximum effective dose is 99.8. The average percentage response corresponding to the break in the latent period curve is 99.1; the differences between the values for the various hydrocarbons are much less than the errors associated with the respective estimates. It is clear, then, that the dose which just produces the minimum latent-period response is also that which just produces the maximum percentage response and that the maximum effective dose (based on latent periods) may be considered as equivalent to about dose TD_{99}. For the three hydrocarbons under consideration, the maximum effective dose is approximately 16 times the dose that produces 50-percent tumors (TD_{50}).

Although the relationship between tumor-incidence response and maximum effective dose was essentially the same for the three materials in the present investigation, further study is necessary before the maximum effective dose (based on latent-period data alone) can be considered as a satisfactory criterion for the comparison of carcinogenic hydrocarbons in general. This criterion is definitely not applicable to another type of tumor-producing agent, the rabbit papilloma virus, of which the dose that just produces the minimal latent-period response is more than 16 times that which just gives the maximum (99-percent) tumor incidence (*19, fig. 8*).

SUMMARY

The quantitative relations between dose and tumor response are studied for the following carcinogenic hydrocarbons and dosages: 20-methylcholanthrene in serial twofold doses of from 0.00024 to 1.0 mg., and 1, 2, 5, 6-dibenzanthracene and 3, 4-benzpyrene both in serial twofold doses ranging from 0.00195 to 8.0 mg.

Both the tumor-incidence and latent-period types of response are analyzed in their relations to dose, by using appropriate biomathematical procedures.

The relative carcinogenic potencies and the relative speeds of action of the three materials are determined for the conditions of the experiment.

Useful relations between dose and response are presented in graphic form, and some implications of the various findings are discussed.

The computed values of certain of the most useful data are as follows (for limits of error of the various estimates, see text): Minimal latent period (average, in months (days/30)): dibenzanthracene 3.72, methcholanthrene 2.48, and benzpyrene 3.02. Median tumor dose (TD_{50})): dibenzanthracene 0.016 mg., methylcholanthrene 0.021 mg., and benzpyrene 0.101 mg. 95-Percent tumor dose (TD_{95}): dibenzanthracene 0.084 mg., methylcholanthrene 0.096 mg., and benzpyrene 08.75 mg. 5-Percent tumor dose (TD_5): dibenzanthracene 0.003 mg., methylcholanthrene 0.0045 mg., and benzpyrene 0.012 mg. Specific induction time in months (days/30): dibenzanthracene 6.79, methylcholanthrene 5.14, and benzpyrene 5.23.

REFERENCES

(1) BRYAN, W. R., and SHIMKIN, M. B.: Quantitative analysis of dose-response data obtained with carcinogenic hydrocarbons. J. Nat. Cancer Inst., 1: 807–833 (1941).

(2) ANDERVONT, H. B.: Production of dibenzanthracene tumors in pure strain mice. Pub. Health Rep., 49: 620–624 (1934).

(3) SHEAR, M. J.: Studies in carcinogenesis. V. Methyl derivatives of 1: 2-benzanthracene. Am. J. Cancer, 33: 499–537 (1938).

(4) SHIMKIN, M. B., and BRYAN, W. R.: The morphology and growth of subcutaneous tumors induced with carcinogenic hydrocarbons in strain C3H mice. J. Nat. Cancer Inst. (To be published later).

(5) BLISS, C. I.: The calculation of the dosage-mortality curve. Ann. Appl. Biol., 22: 134–167 (1935).

(6) ———: The comparison of dosage-mortality data. Ann. Appl. Biol., 22: 307–333 (1935).

(7) ———: The determination of the dosage-mortality curve from small numbers. Quart. J. Pharm. & Pharmacol., 11: 192–216 (1938).

(8) IRWIN, J. O.: Statistical method applied to biological assays. Suppl. to J. Roy. Statis. Soc., 4: 1–60 (1937).

(9) SHIMKIN, M. B., and ANDERVONT, H. B.: Comparative carcinogenicity of three carcinogenic hydrocarbons. J. Nat. Cancer Inst., 1: 57–62 (1940).

(10) LETTINGA, T. W.: De carcinogene wurking van kleine doses 1, 2, 5, 6-dibenzanthraceen. Academisch Proefschrift, Amsterdam, Van Gorcum & Co., Assen (1937).

(11) SHIMKIN, M. B.: Biologic testing of carcinogens. I. Subcutaneous injection technique. J. Nat. Cancer Inst., 1: 211–223 (1940).

(12) GADDUM, J. H.: Methods of biological assay depending on the quantal response. M. Research Council, Spec. Rep. Ser. No. 183. H. M. Stationery Office, London (1933).

(13) BOYLAND, E., and WARREN, F. L.: The induction of tumours by methylcholanthrene in two strains of mice. J. Path. & Bact., 45: 171–177 (1937).

(14) FIESER, L. F.: Carcinogenic activity, structure, and chemical reactivity of polynuclear aromatic hydrocarbons. Am. J. Cancer, 34: 37–124 (1938).

(15) GOTTSCHALK, R. G.: Quantitative studies on tumor production in mice by benzpyrene. Proc. Soc. Exper. Biol. & Med., 50: 369–373 (1942).

(16) LEITER, J., and SHEAR, M. J.: Quantitative experiments on the production of subcutaneous tumors in strain A mice with marginal doses of 3, 4-benzpyrene. J. Nat. Cancer Inst., 3: 455–477 (1943).

(17) ANDERVONT, H. B., and SHIMKIN, M. B.: Biologic testing of carcinogens. II. Pulmonary-tumor-induction technique. J. Nat. Cancer Inst., 1: 225–239 (1940).

(18) FOSTER, R. H. K.: Standardization of safety margin. J. Pharmacol. & Exper. Therap., 65: 1–17 (1939).

(19) BRYAN, W. R., and BEARD, J. W.: Studies on the purification and properties of the rabbit-papilloma-virus protein. J. Nat. Cancer Inst., 1: 607–673 (1941).